November 11, 1999, in accordance with the terms and provisions of the Budapest Treaty relating to the deposit of microorganism. FA112 and FA113 have been assigned ATCC Accession No. PTA-938 and PTA-939, respectively.

Please replace the paragraph at page 4, lines 4-9, with the following replacement paragraph:

The host cell can comprise a nucleic acid encoding a catalyst of disulfide bond isomerization, e.g., variants of a thioredoxin or glutaredoxin, which have, e.g., a redox potential that is higher than that of its wild type counterpart. In an illustrative example, the variant is a "Grx" variant of thioredoxin A. The host cell can also comprise a catalyst of disulfide bond isomerization, such as a disulfide bond isomerase, e.g., DsbC, or derivative thereof.

Please replace the two paragraphs at page 5, lines 32-38, with the following replacement paragraphs:

Figure 8B is an alignment of amino acid sequences of AhpC proteins from different microorganisms and from the human species (HUMAN_TSA). The numbers represent the amino acid position of the first amino acid shown in each protein. The sequences correspond, from top to bottom, to SEQ ID Nos: 12-20.

Figure 9 is a diagram showing the two different forms of AhpC that can be found in a cell depending on the oxidative stress-inducing signal. The form on the left represents the wild-type enzyme and the form on the right, the mutant enzyme.

Please replace the paragraph at page 6, lines 20-40, with the following replacement paragraph:

Host cells or organisms of the invention for the efficient production of disulfide bond containing proteins can be produced by various modifications or combinations of modifications of wild type cells or organisms or cells or organisms which have already been modified. In one embodiment, a host cell is modified by reducing or eliminating the level or activity of one or more reductases in the host cell. In a preferred embodiment, the reductase is selected from the group consisting of the thioredoxin reductase (trxB); glutathione (gshA and gshB); and the glutathione oxidoreductase (gor). Such a host cell can further be modified to increase its rate of growth, if necessary, such as selecting naturally occurring mutants, e.g., suppressor mutants, or by the introduction of a mutation or a heterologous DNA or stimulating the expression or activity of a gene, thereby resulting in an increased growth rate of the host cell. A modification of a host cell resulting in improved growth is referred to herein as "growth inducing modification." Growth of modified host cells can be improved or restored to that of wild type host cells by increasing the reducing environment of the cytoplasm, preferably without affecting the oxidative environment necessary for appropriate oxidation of disulfide bond containing proteins. Accordingly, the

oxidizing role of the thioredoxins in the host cell is preferably not modified. In one embodiment, a modified host cell is modified by altering the activity of the AphC subunit of the alkyl hydroperoxidase AhpCF, such as by mutating the region of the aphC gene containing four TCT triplets, so that the enzyme has a new reducing activity. A preferred *E. coli* bacterial strain having a mutated aphC gene is the strain FA113 which has been deposited at the ATCC and has been assigned ATCC Accession No. PTA-939.

Please replace the paragraph at page 10, lines 34-39, with the following replacement paragraph:

The term "disulfide bond isomerization" refers to an exchange of disulfide bonds between different cysteines, i.e., the shuffling of disulfide bonds (see Figure 1). Isomerization of disulfide bonds is mediated by thiol-disulfide exchange between the active site cysteines of enzymes and cysteines in the target protein (see Figure 1) and catalyzed by isomerases. In *E. coli*, isomerization is catalyzed by DsbC, a periplasmic disulfide bond oxidoreductase.

Please replace the paragraph at page 11, lines 1-8, with the following replacement paragraph:

"Protein disulfide bond isomerases" refer to proteins which catalyze the isomerization of disulfide bonds in proteins. Without wanting to be limited to a specific mechanism of action, isomerases are thought to act initially by invading incorrect disulfide bonds that have been formed in proteins and then allowing or promoting isomerization of the disulfide bond. To carry out this process, it is posited that the two cysteines in the Cys-Xaa-Xaa-Cys motif must be in the reduced state (Figure 1). In fact, DsbC is found with its active site cysteines in the reduced state in wild-type *E. coli*. DsbC is maintained in a reduced state in a cell by the cytoplasmic membrane protein DsbD (or DipZ protein).

Please replace the paragraph at page 11, lines 18-26, with the following replacement paragraph:

The term "thioredoxin fold" refers to an overall protein structural motif that is shared by the members of the thioredoxin superfamily. Thus, although thioredoxins and glutaredoxins may have relatively different amino acid sequences, they share a similar secondary structure, i.e., a similar overall fold, referred to as the thioredoxin fold. The thioredoxin fold consists of a central four-stranded beta-sheet flanked by three alpha-helices (see, e.g., Figure 1 in Jordan et al. (1997), *J. Bio. Chem.* 272:18044). The thioredoxin fold has been found in five distinct classes of proteins that have the common property of interacting with cysteine-containing substrates (see, e.g., Martin J.L.(1995) *Structure* 3: 245 and Aslund et al. (1996) *J. Biol. Chem.* 271:6736).

Please replace the paragraph at page 12, lines 23-25, with the following replacement paragraph:

Q8

"DsbB," which is encoded by the gene *dsbB*, is a cytoplasmic membrane protein which oxidizes DsbA. DsbB contains a Cys-Xaa-Xaa-Cys (SEQ ID NO: 1) (Xaa being any amino acid residue) motif. DsbB may be oxidized by passing electrons to the respiratory chain.

Please replace the paragraph bridging pages 15 and 16, with the following paragraph:

Q9

Suitable bacteria for this purpose include archaebacteria and eubacteria, especially *eubacteria*, and most preferably *Enterobacteriaceae*. Other examples of useful bacteria include *Escherichia, Enterobacter, Azotobacter, Erwinia, Bacillus, Pseudomonas, Klebsiella, Proteus, Salmonella, Serratia, Shigella, Rhizobia, Vitreoscilla, and Paracoccus*. Suitable *E. coli* hosts include *E. coli* DHB4, *E. coli* BL-21 (which are deficient in both Lon (Phillips et al. (1984) *J. Bacteriol.* 159: 283.) and OmpT proteases), *E. coli* AD494, *E. coli* W3110 (ATCC 27,325), *E. coli* 294 (ATCC 31,446), *E. coli* B, and *E. coli* X1776 (ATCC 31,537). Other strains include *E. coli* B834 which are methionine deficient and, therefore, enables high specific activity labeling of target proteins with ^{35}S-methionine or selenomethionine (Leahy et al. (1992) *Science* 258, 987). Yet other strains of interest include the BLR strain, and the K-12 strains HMS174 and NovaBlue, which are recA- derivative that improve plasmid monomer yields and may help stabilize target plasmids containing repetitive sequences (these strains can be obtained from Novagen).

Please replace the paragraph page 34, lines 26-39, with the following paragraph:

Q10

In an illustrative embodiment, disulfide bond containing proteins of the invention are produced as follows. A host cell or organism of the invention is first transformed with an expression plasmid encoding a polypeptide of interest and a selection marker. The plasmid can encode additional polypeptides, such as is desired, e.g., in the production of multi-polypepeptide proteins. Additional plasmids encoding other polypeptides can be co-transformed, or transformed separately into the host cell or organism. When using more than one plasmid, it may be preferable to use different markers of selection, to insure that all the desired plasmids are contained in the recombinant host cell that is selected. Following transformation of the one or more plasmids into the host cells, according to known methods, clones having taken up the plasmid(s) are selected on appropriate medium, and cloned. Separate clones are then tested to confirm that they have the desired characteristics, including the expression of the one or more polypeptides. In particular, the polypeptide(s) of interest can be isolated from the host cells, and tested for activity, amount, etc. The isolated clones can then be frozen in aliquots for preservation, pursuant to methods well known in the art.

Please replace the paragraph bridging pages 38-39, with the following paragraph:

A protein of the invention be used in one or more of the following purposes or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or bodypart size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

Q11

Please replace the paragraph at page 43, lines 1-7, with the following replacement paragraph:

Q12

For establishing the growth curve of the FA113 strain and compare it to its wild type parent strain, the bacteria were subjected to aerobic growth at 37°C in LB medium in test tubes. The results, which are presented in Figure 4, show that at 37°C in rich media, FA113 was found to grow almost as well as the wild type (DHB4, trxB+ gor+) strain with doubling times 30 and 27 minutes, respectively. In contrast, WP778, the trxB gor parent of FA113 grew with a doubling time of 300 min in the absence of DTT (Prinz, et al. (1997) *J. Biol. Chem.* 272: 15661).

Please replace the paragraph at page 46, lines 10-11, with the following replacement paragraph:

Q13

Thus, properly formed and oxidized alkaline phosphatase forms in the cytoplasm of the trxB gor supp mutant FA113.

Please replace the paragraph at page 46, lines 15-28, with the following replacement paragraph:

Stewart et al. (Stewart, et al. (1998) *EMBO J.* 17: 5543) have shown that disruption of trxB results in an accumulation of oxidized thioredoxins which can then act as oxidases, the reverse of their normal role. Likewise, in FA113, TrxA expressed from the chromosome was present solely in the oxidized form. We examined the effect of high level expression of TrxA and TrxA mutant proteins with varying redox potentials on the folding of the more complex multi-disulfide proteins, namely vtPA and tPA. The redox potential of most cysteine oxidoreductases, including TrxA, is strongly influenced by the sequence of the dipeptide within the CXXC (SEQ ID NO: 1) active site motif (Mossner, et al. (1999) *J. Biol. Chem.* 274: 25254; Mossner, et al. (1998) *Protein Sci.* 7: 1233; Grauschopf, et al. (1995) *Cell* 83: 947). TrxA with a wild type active site (-CGPC-; SEQ ID NO: 2) and five mutants with varying redox potentials (see below) were cloned into plasmid pBAD33 (Guzman et al. (1995) *J. Bacteriol.* 177:4121) under the control of the araBAD promoter and transformed into FA113 together with a compatible expression vector for vtPA or full length tPA synthesis.

Please replace the paragraph at page 47, lines 27-30, with the following replacement paragraph:

Thus, the results of this Example show that cotransformation of TrxB gor supp mutant with a plasmid encoding a thioredoxin variant having a higher redox potential than its wild type counterpart significantly increases the production of proteins containing multiple disulfide bonds in the cytoplasm of these cells.

Please replace the paragraph at page 50, lines 27-32, with the following replacement paragraph:

The open reading frames coding for ahpCF were amplified from the chromosome of the wild type and the FA113 mutant by PCR and cloned into the pACYC derivative pLAC-YC. Constructs containing either the entire operon or just the *ahpC*-gene (wild type or mutant) were transformed into JL10 and DR456, in which in addition to the *trxB* and *gor* mutations, the *ahpCF* locus is also inactivated. DR456 is also referred to as "trxB gor ahpCF::Km mutant". Growth of each of these strains was determined on rich medium (NZ).

Please replace the paragraph at page 53, lines 18-24 with the following replacement paragraph:

Relevant genotype	Growth on rich medium
TrxB gor	No